Phloroglucinol 1 (Scheme 1) is found as a substituent in a variety of natural products. However, biosynthesis of phloroglucinol 1 as a free-standing molecule has not been delineated. As part of a search for such biosynthetic activity, biosynthesis of acetylphloroglucinols encoded by the phlACBDE gene cluster found in *Pseudomonas fluorescens* Pf-5 was examined. 1 In addition to 2,4-diacetylphloroglucinol 7 and 2-acetylphloroglucinol 6 (Scheme 1), formation of phloroglucinol 1 was detected. Subsequent heterologous expression of phlD led to accumulation of phloroglucinol 1 in *Escherichia coli* cultures. PhlD-catalyzed formation of phloroglucinol 1 suggests an alternative to the previously proposed route for the biosynthesis of acetylphloroglucinols. 2 In addition, PhlD activity expressed by intact microbes provides the basis for the formulation of new syntheses (Scheme 2) of phloroglucinol 1 and resorcinol 11.

The condensation of three malonyl-CoA molecules required for the biosyntheses of phloroglucinol 1 and triacetic acid lactone 3 (Scheme 1) may differ only in the timing of a single decarboxylation. Decarboxylation of the priming malonyl-CoA may lead to 3,5-diketohexanoate 2a (Scheme 1). 11 A stabilized C-4,5 enolate in 2b may cyclize to triacetic acid lactone 3, while decarboxylation of 2a and cyclization of a C-6 carbanion may lead to phloroglucinol 1. Triacetic acid lactone 3 has been synthesized by *Gerbera hybrida* 2-pyrene synthase, 2 mutated *Brevibacterium ammoniagenes* fatty acid synthase B, 2 and mutated *Penicillium patulum* 6-methylsalicylic acid synthase. 3 Phloroglucinol was not formed by any of these enzymes.

Prospecting for the biosynthesis of phloroglucinol 1 led to *P. fluorescens* Pf-5 and the biosynthesis of 2,4-diacetylphloroglucinol 7 (Scheme 1). 1 Acetylphloroglucinol biosynthesis is encoded by a gene cluster consisting of phlACBD, a protein for product export encoded by phlE, 2 and a divergently transcribed phlF-encoded regulator. PhlD has been suggested to be involved in the formation and cyclization of an activated 3,5,7-triketooctanoate 5 (Scheme 1). 1

The resulting intermediate 2-acetylphloroglucinol 6 is then presumably acetylated to form 2,4-diacetylphloroglucinol 7 (Scheme 1). 2 Biosynthesis of phloroglucinol 1 is not an activity that has been assigned to PhlD.

*P. fluorescens* Pf-5/pME6031 was examined for products that accumulated in its culture supernatants. In addition to accumulation of 2,4-diacetylphloroglucinol 7 and 2-acetylphloroglucinol 6, formation of phloroglucinol 1 was discovered (entry 1, Table 1). To increase the concentration of biosynthesized phloroglucinols, 1 *P. fluorescens* Pf-5 was transformed with pJA2.232, a plasmid derived from the insertion of the phlACBDE gene cluster into pME6031. The goal was to evade regulation by genomically encoded PhlF by presenting multiple copies of the biosynthetic gene.

Scheme 1

(a) Biosynthesis of acetylphloroglucinols 6 and 7 via phloroglucinol 1. (b) Previously proposed biosynthesis of acetylphloroglucinols 6 and 7. (c) Biosynthesis of triacetic acid lactone 3.

Scheme 2

(a) *Na₂Cr₂O₇*, *H₂SO₄*. (b) *Fe*, *HCl*. (c) *H₂SO₄*, 108 °C; (d) see ref 4; (e) Dowex 50 H⁺, *MeOH*. (f) *Na*, *MeOH*. 185 °C; (g) 12 N *HCl*. (h) phlD-expressing microbe: (i) *H₂*, *Rh* on *Al₂O₃*, ii. 0.5 M *H₂SO₄*, reflux; (j) *SO₃*, *H₂SO₄*. (k) *NaOH*, 350 °C; (l) HZSM-12, propene; (m) i. *O₂*, ii. *H₂O₂*, iii. *H⁺*.

This approach resulted in large increases in the concentrations of synthesized phloroglucinols 1, 6, and 7 (entry 2 vs entry 1, Table 1). Further analysis followed from heterologous expression from a T7 promoter of phlACBDE genes in *Escherichia coli* (entry 3–7, Table 1). All *E. coli* constructs also carried a chromosomal gene1 insert encoding the T7 RNA polymerase. *E. coli* BL21(DE3)/pJA3.085, which carried a phlACBDE plasmid insert, synthesized phloroglucinol 1 and 2-acetylphloroglucinol 6 but no 2,4-diacetylphloroglucinol 7 (entry 3, Table 1). The absence of the phlE-encoded product exporter in *E. coli* BL21(DE3)/pJA3.156 had only a modest impact on the concentrations of biosynthesized phloroglucinol 1 and 2-acetylphloroglucinol 6 (entry 4, Table 1). Product formation attendant with heterologous expression of only phlD was
then evaluated using *E. coli* fermentor-controlled conditions was examined using *E. coli* (DE3)/pJA3.131A (entry 6, Table 1). Under these culture conditions, *P. fluorescens* Pf-5, which carried plasmid-localized phlACB, was employed as a substrate. Approximately equal specific activities of the experiments summarized in Table 1.

**Table 1. Maximum Concentrations of Phloroglucinol 1, 2-Acetylphloroglucinol 6, and 2,4-Diacetylphloroglucinol 7 Biosynthesized by Constructs Expressing phlACBDE Genes**

<table>
<thead>
<tr>
<th>entry</th>
<th>host/ plasmid</th>
<th>plasmid inserts</th>
<th>phloroglucinols (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>P. fluorescens</em> Pf/ pME6031&lt;sup&gt;a&lt;/sup&gt;</td>
<td>none</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td><em>P. fluorescens</em> Pf/ pJA2.232&lt;sup&gt;b&lt;/sup&gt;</td>
<td>phlACBDE</td>
<td>470</td>
</tr>
<tr>
<td>3</td>
<td>E. coli BL21(DE3)/ plJA3.085&lt;sup&gt;c&lt;/sup&gt;</td>
<td>phlACBDE</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>E. coli BL21(DE3)/ plJA3.156&lt;sup&gt;d&lt;/sup&gt;</td>
<td>phlACB</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>E. coli BL21(DE3)/ plJA2.042&lt;sup&gt;e&lt;/sup&gt;</td>
<td>phlD</td>
<td>720</td>
</tr>
<tr>
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<td>E. coli JWF1(DE3)/ plJA3.131A&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>780</td>
</tr>
<tr>
<td>7a</td>
<td><em>E. coli</em> BL21(DE3)/ plJA3.169</td>
<td>phlACB</td>
<td>0&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
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<td>phlACB</td>
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<tr>
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</tr>
<tr>
<td>7d</td>
<td><em>P. fluorescens</em> Pf/ pME6031&lt;sup&gt;a&lt;/sup&gt;</td>
<td>none</td>
<td>0&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cells were cultured in YM medium under shake-flask conditions.  
<sup>b</sup> Cells were cultured under shake-flask conditions in TB medium and harvested. Following resuspension in M9 minimal salts medium, cells were cultured under shake-flask conditions.  
<sup>c</sup> Cells were cultured in M9 minimal salts medium under fermentor-controlled conditions. Concentrations of phloroglucinols 48 h after addition of 1 (100 mg/L), 6 (100 mg/L), or 7 (100 mg/L) to cells cultured in M9 medium under shake-flask conditions.

PhlD is of particular importance in establishing the outline of new syntheses of phloroglucinol 1 and resorcinol 11 (Scheme 2). Phloroglucinol is currently synthesized (Scheme 2) from 2,4,6-trinitrotoluene 8 by a route involving an oxidation utilizing Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub>. Beyond the explosion hazard, environmentally problematic chromates are generated along with other salts as waste streams during synthesis of phloroglucinol 1 from 2,4,6-trinitrotoluene 8. Recently, an alternate route (Scheme 2) to phloroglucinol 1 has been elaborated involving microbe-catalyzed synthesis of triacetic acid lactone 3. Multiple chemical steps are needed to convert triacetic acid lactone 3 into phloroglucinol 1 via intermediacy of the methyl ethers 9 and 10 (Scheme 2). In contrast to these chemical and chemoenzymatic routes to phloroglucinol, heterologous expression of PhlD in *E. coli* allows phloroglucinol 1 to be made in a single microbe-catalyzed step from glucose (Scheme 2).

Resorcinol 11 is currently manufactured (Scheme 2) by alkali fusion of 1,3-benzenedisulfonic acid 12 or hydroperoxidation of 1,3-diisopropylbenzene 13. Alkali fusion requires high temperatures and generates large salt waste streams. Acetone hydroperoxide formed during hydroperoxidation is an explosion hazard. In addition, both 1,3-benzenedisulfonic acid 12 and 1,3-diisopropylbenzene 13 are produced from petroleum-derived, carcinogenic benzene (Scheme 2). The new route to resorcinol 11 is based on the Rh-catalyzed hydrogenation<sup>(2)</sup> (Scheme 2) of microbe-synthesized phloroglucinol 1. Acid-catalyzed dehydration of the resulting dihydroresorcinol intermediate leads to resorcinol 11. Since phloroglucinol 1 can now be synthesized from glucose, resorcinol joins catechol<sup>9</sup> and hydroquinone<sup>10</sup> as a dihydroxy aromatic that is amenable to synthesis from nontoxic, plant-derived glucose (Scheme 2).

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**Supporting Information Available:** Plasmid maps; strain construction; culture conditions; enzyme assays; pH optimum for PhlD activity (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

**References**